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Presence of Basal Lamina-like Substance with Anchoring Fibrils Within the Amyloid Deposits of Primary Localized Cutaneous Amyloidosis

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The dermal-epidermal (DE) junction areas of skin specimens obtained from 16 patients with either lichen amyloidosis or macular amyloidosis were studied. In the dermal papillae where amyloid was deposited, elastic fibers frequently were absent, but periodic acid-Schiff reaction after diastase digestion was homogenously positive. Ultrastructural studies revealed that a basal lamina-like substance with anchoring fibrils was present between and within amyloid deposits. By indirect immunofluorescence technique using an anti-basement membrane zone antiserum obtained from a patient with bullous pemphigoid, specific linear fluorescence occurred at the DE junction, and in a reticular pattern in dermal papillae. It seemed that apoptotic keratinocytes of the epidermis brought down basal lamina and fine fibrous components attached to it when these cells dropped down to the papillary dermis and became the source of amyloid. These findings support the hypothesis that epidermal keratinocyte degeneration plays an important role in the histogenesis of cutaneous amyloidoses.

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Abbreviations:
BP-antibody: antiserum to the basement membrane zone of the skin
DE: dermal-epidermal
FITC: fluorescein isothiocyanate
O & G: orcein and Giemsa
PAS: periodic acid-Schiff
PBS: phosphate-buffered saline at pH 7.2

Filamentous degeneration of epidermal keratinocytes seems to play an important role in the histogenesis of lichen amyloi-

dosis and macular amyloidosis [1–5]. The sequential changes from epidermal keratinocytes to amyloid have been demonstrated on a morphologic base [2–5]. A recent advance made by the anti-keratin antibody technique [6,7] also suggests that amyloid of primary localized cutaneous amyloidosis has the same antigenicity to a certain epidermal fibrous protein.

In this study, we add new evidence to support the hypothesis that amyloid in organ-limited cutaneous amyloid derives from apoptotic keratinocytes which drop off through the dermal-epidermal (DE) junction into the upper dermis [2–4].

TABLE I. *Clinical data of Japanese patients*

No.	Diagnosis	Age	Sex	Distribution	Duration (years)
1	Lichen amyloidosis	47	M	Back, legs	27
2	Macular amyloidosis	46	F	Back	0.5
3	Macular amyloidosis	60	M	Back	40
4	Macular amyloidosis	21	F	Back	several
5	Macular amyloidosis	60	M	Back	0.5
6	Macular amyloidosis	50	F	Back	...
7	Macular amyloidosis	30	F	Back	5
8	Lichen amyloidosis	38	M	Back, legs	5

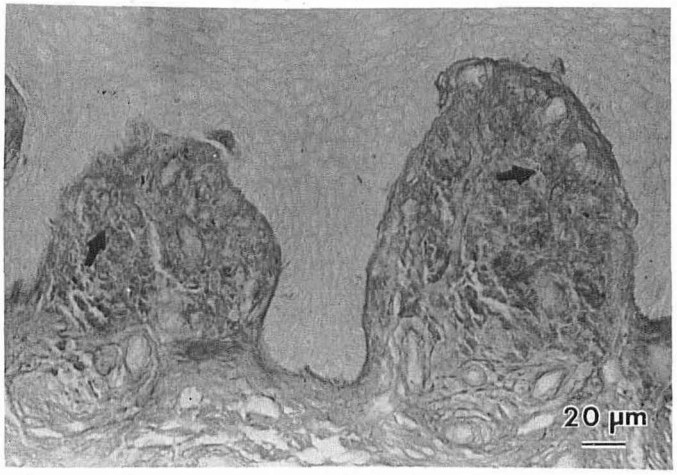

FIG 1. PAS reaction shows positive reaction at the DE junction area as well as in the papillary dermis. PAS-positive substance is either linear or reticular (*arrows*) and is not exactly the amyloid itself. PAS with diastase digestion.

MATERIALS AND METHODS

Subjects

In addition to 8 patients who were studied in our previous report [3], 2 Japanese patients with lichen amyloidosis and 6 Japanese patients with macular amyloidosis were examined (Table I). The biopsy specimens obtained from the skin lesions of all patients were prepared for histologic and electron microscopic studies. Four of the skin specimens from the Japanese patients were cut into 3 pieces, one of which was immediately frozen for immunohistochemical studies.

Histologic Studies

All specimens were fixed in neutral formaldehyde or Bouin's solution and processed by our routine method [3]. Amyloid was demonstrated by crystal violet, Congo red, and thioflavin T stains. Polarizing microscope examination revealed green birefringence in the dermal papillae, where amyloid was observed by other staining methods. Eight specimens from the new patients were also stained with orcein and Giemsa (O & G) and were examined by periodic acid-Schiff (PAS) reaction with or without previous digestion with diastase.

Ultrastructural Studies

The skin specimens were fixed in 5% glutaraldehyde and 1% osmium tetroxide. Our routine method [3] was performed for dehydration, embedding, and sectioning. The thin sections were double-stained with uranyl acetate and lead citrate [8]. Hitachi HU12A and H300 electron microscopes were used for observation and photography.

Immunohistochemical studies

The frozen specimens were cut into 4 μm-thick sections using a cryostat. Antiserum to the basement membrane zone of the skin (BP-antibody) was obtained from a 65-year-old woman with bullous pemphigoid. The antiserum, with maximum positive dilution titer of 128, was diluted 1:10 with phosphate-buffered saline (PBS) at pH 7.2.

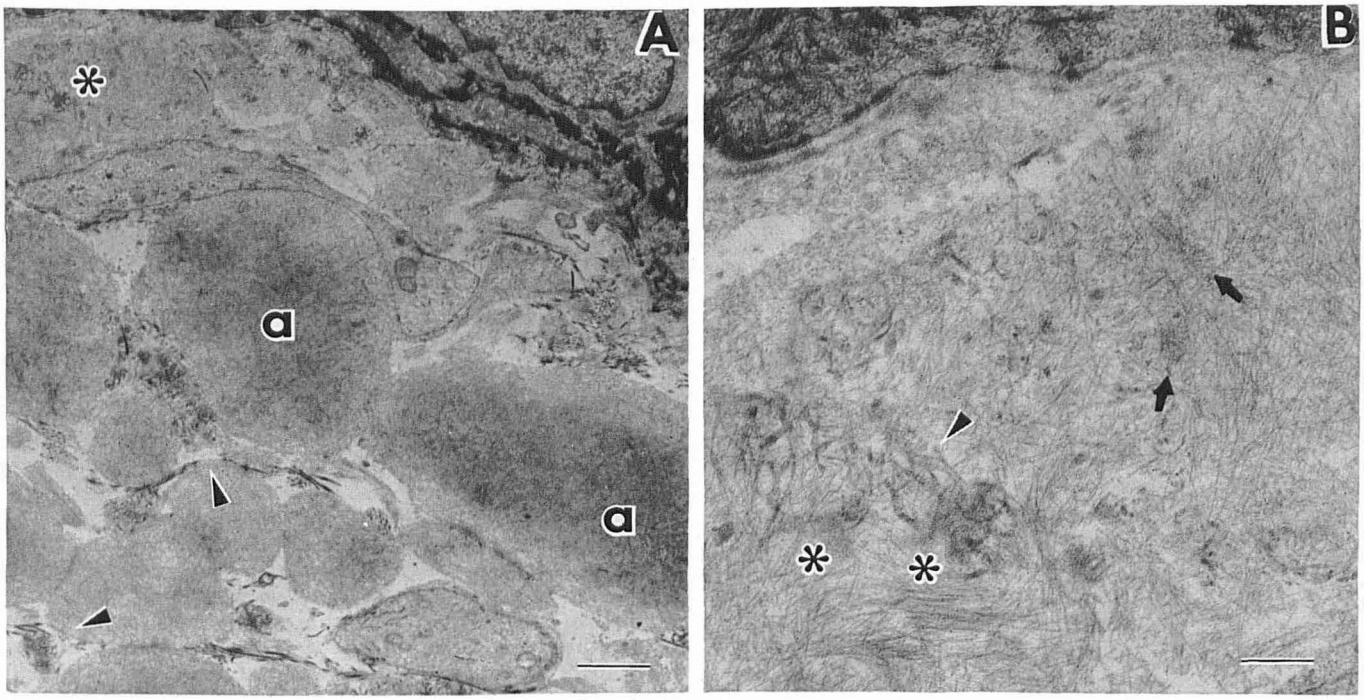

FIG 2. *A*, Between amyloid islands (*a*) in a dermal papilla, there are flocculent materials (*arrowheads*), one of which (*asterisk*) is located between amyloid masses. *Bar* = 1 μm. *B*, Enlargement of the area marked by the *asterisk* in Fig 2*A*. Basal lamina is thin and focally absent. Anchoring fibrils are not attached to the basal lamina but are admixed with amyloid filaments (*arrowhead*). Basal lamina-like materials are seen within the aggregation of these fibrils (*asterisks*) as well as independently (*arrows*). *Bar* = 200 nm.

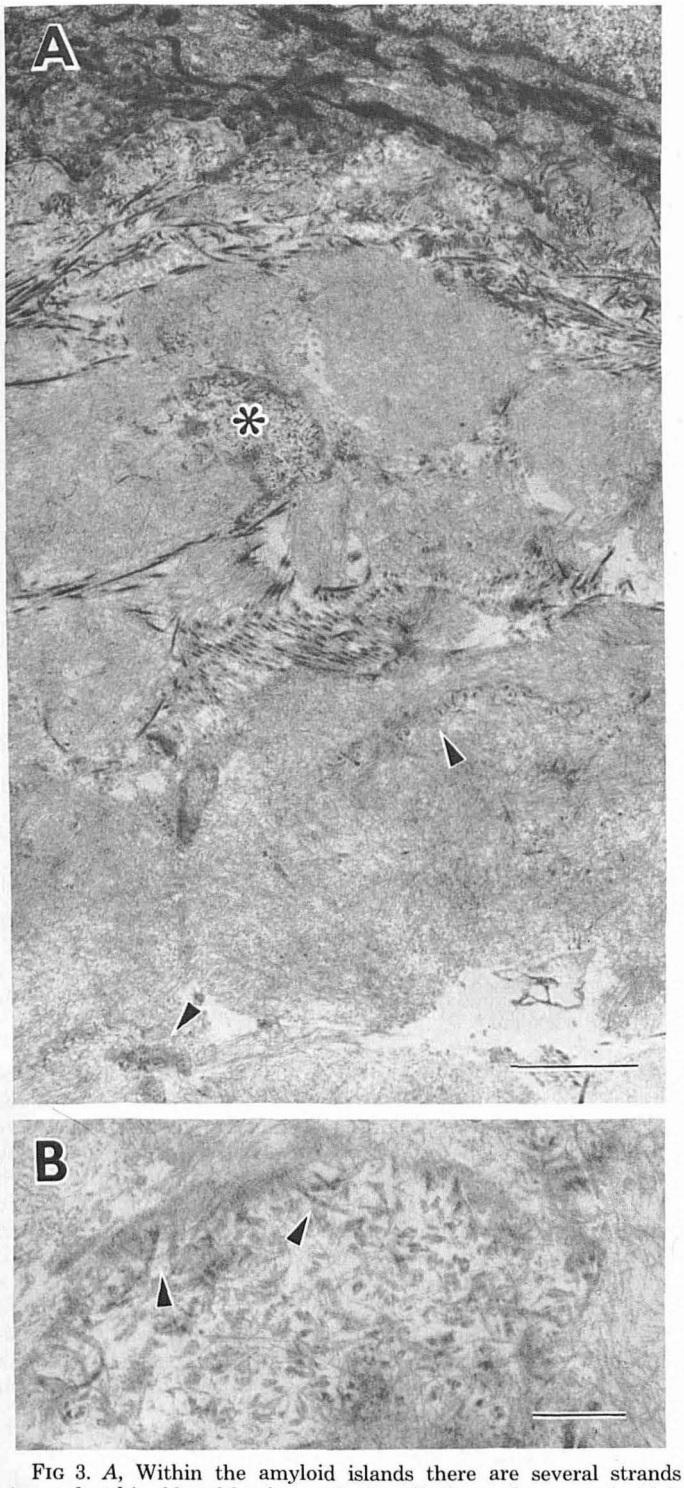

FIG 3. *A*, Within the amyloid islands there are several strands (*arrowheads*) of basal lamina-anchoring fibril complex, one of which (*asterisk*) borders amyloid masses. *Bar* = 1 μm. *B*, The strands are revealed to be an admixture of basal laminae and anchoring fibrils which show a characteristic irregular banding pattern (*arrowheads*). *Bar* = 200 nm.

Antiserum for indirect immunofluorescence was prepared as follows: fluorescein isothiocyanate (FITC)-conjugated rabbit antiserum to human IgG (Behring Institute, West Germany) was adjusted to a fluorescein to protein ratio of less than 2.0 to avoid nonspecific staining due to high concentration of fluorescein.

Frozen sections were fixed for 5 min in 90% ethanol and 10 min in 70% ethanol. After washing in PBS for 5 min at 20°C, they were incubated with BP-antibody for 30 min at 37°C in a moist chamber. After washing in PBS for 15 min, they were incubated with FITC-labeled antiserum to human IgG, IgA, IgM, C1q, or C3 for 30 min at 37°C in a moist chamber. As a blocking test, selected specimens incubated with BP-antibody were incubated with antihuman IgG without FITC conjugation for 30 min at 37°C in a moist chamber prior to incubation with the FITC-labeled antihuman IgG.

After a 15-min washing in PBS, the sections were mounted in a 9:1

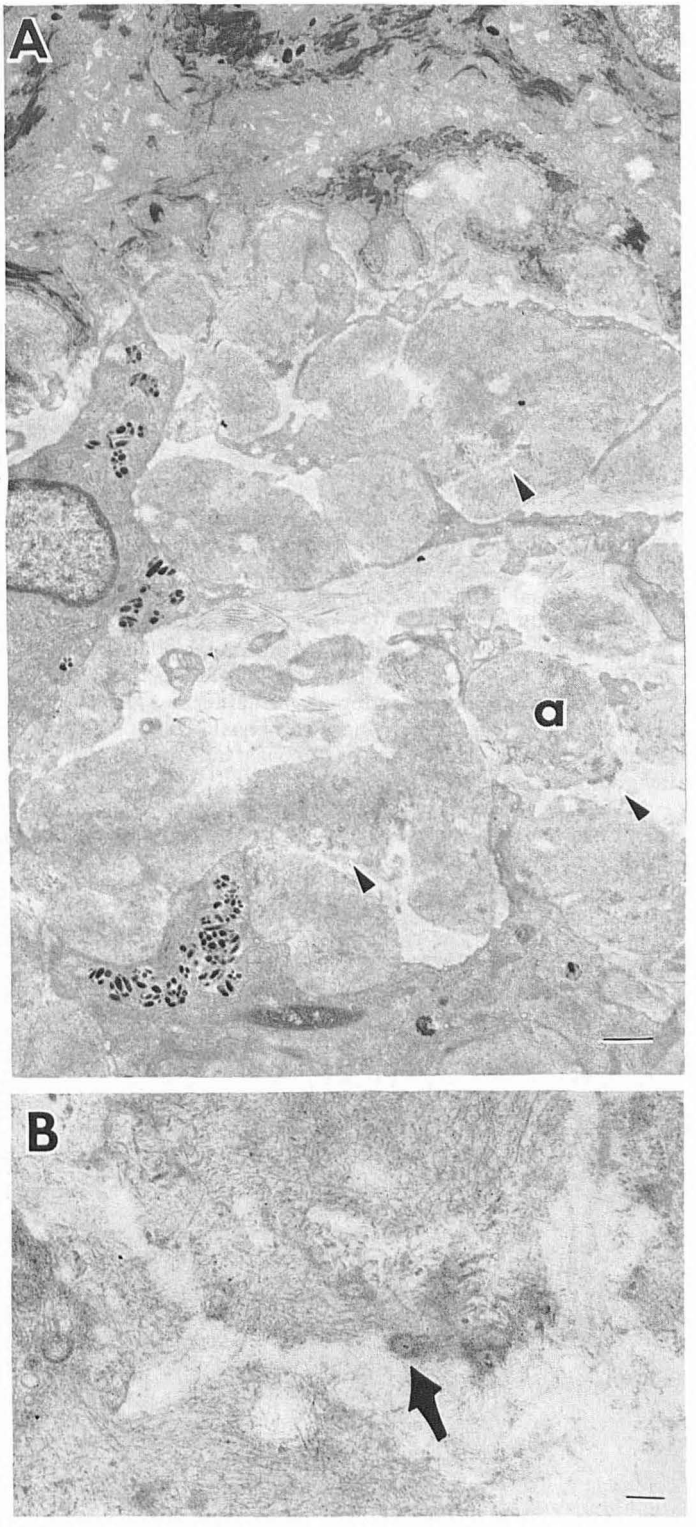

FIG 4. *A*, Many amyloid islands (*a*) are seen in a dermal papilla. There are several areas of electron-dense flocculent material (*arrowheads*). *Bar* = 1 μm. *B*, A higher magnification of the area marked by *a* in Fig 4*A* shows cross-section of an anchoring fibril surrounded by a halo, which looks like a bull's eye (*arrow*). *Bar* = 200 nm.

mixture of glycerin:PBS. Photographs were taken under a fluorescence microscope (Olympus AH-RFL-LB type) with a super-high-pressure mercury bulb as the light source, fitted with DM 400, L 420, and UG 1 filters. After photographing in fluorescence microscopy, the same specimens were stained with crystal violet to confirm the presence of amyloid.

RESULTS

Histologic Studies

All specimens showed similar features: In the dermal papillae, amyloid deposits were demonstrated by crystal violet, thioflavin T, and Congo red stains. O & G technique revealed that elastic fibers were focally reduced or absent at the DE junction area. Particularly, the normal network of elastic fibers under the basement membrane was frequently absent. PAS reaction was positive at the DE junction area as well as in the papillary dermis, where the reaction pattern was reticular (Fig 1). Step sections confirmed that discrete, round masses of amyloid occupied a part of the area where the PAS reaction was positive and elastic fibers were absent. PAS-positive substance, either linear or reticular (Fig 1), was not exactly the amyloid itself because amyloid was a round mass as demonstrated with crystal violet.

Ultrastructural Studies

There were many amyloid islands (Fig 2*A*) in the dermal papillae in all cases examined. The amyloid consisted of nonbranching, straight filaments ~7 nm in diameter.

The DE junction area was extensively studied. In most areas, basal lamina ~70 nm in width continuously invested the dermal aspect of basal cells. Occasionally, however, the basal lamina was thin or focally absent where the attachment of anchoring fibrils, which were characterized by branching short fibrils with several irregular cross-bandings, could not be found on the dermal side (Fig 2*B*). Pseudopods of epidermal keratinocytes protruded into the dermis where the basal lamina was absent. Duplication or multiplication of the basal lamina was also found in some areas.

A striking finding was the presence of a basal lamina-like substance which was almost always associated with anchoring fibrils and found between and/or within the amyloid islands (Figs 2–5). As demonstrated in Fig 2*A*, these basal lamina-anchoring fibril complexes could be located at quite a distance from the DE junction; this ruled out the possibility that they were extensions of duplicated basal laminae that were tangentially sectioned. They were seen also among the collagen fibrils

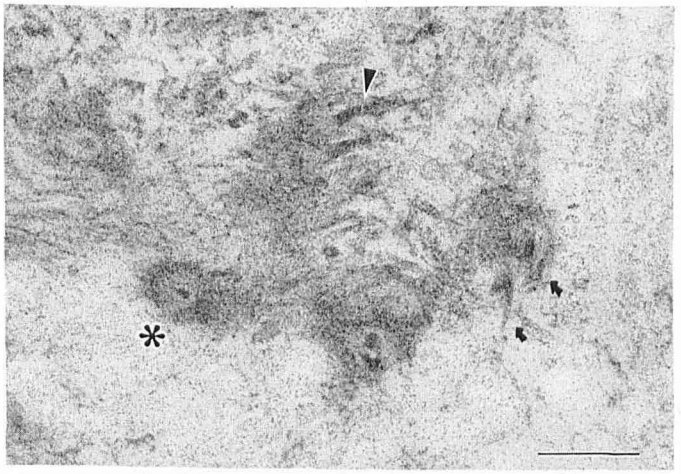

FIG 5. A still higher magnification of the area marked by *a* in Fig 4*A* shows the details: A longitudinal section of anchoring fibrils reveals their insertion into a swollen fragment of basal lamina (*arrowhead*). At the point of insertion there seems to be an electron-lucent space surrounding the fibrils, which can best be seen in semitangentially sectioned fibrils (*arrows*). In cross-section this lucent space forms a halo surrounding the fibrils (*asterisks*). *Bar* = 200 nm.

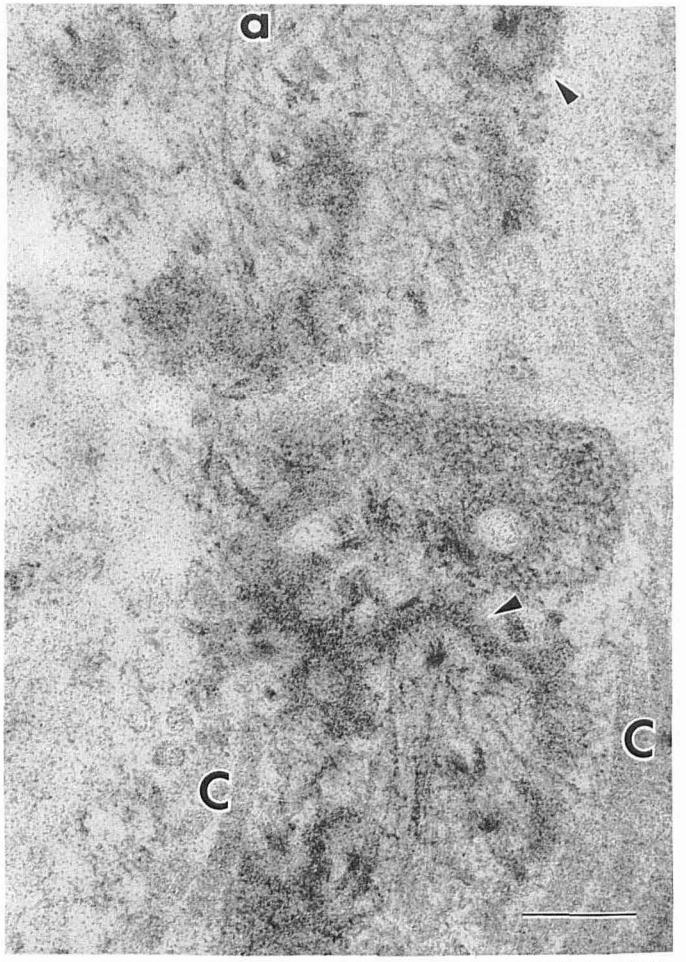

FIG 6. "Bull's eye" structures (*arrowheads*) intermingled with the characteristic amyloid filaments (*a*) are surrounded by collagen fibrils (*C*). *Bar* = 200 nm.

near the amyloid deposits (Fig 6). The basal lamina-like substance had the same electron density and the same amorphous appearance as the basal lamina at the DE junction. They were flocculent material (Figs 2, 4–6) or were forming branching strands (Fig 3). Their width was up to ~35 nm and the length varied from 1–2 μm. The attachment of the characteristic anchoring fibrils was the definite evidence of their similarity to basal laminae. When the insertion area of anchoring fibrils into the swollen, irregular basal lamina-like substance was cut at a right angle to the fibrils, a less dense halo-like ring surrounded the fibrils (Fig 5); the black dot in the center was the cross-section of the anchoring fibril (Fig 5). The basal lamina-like substance with anchoring fibrils was admixed with collagen bundles and amyloid filaments (Fig 6).

Immunohistochemical Studies

All specimens examined by the indirect method using BP-antibody revealed specific immunofluorescence at the DE junction area. There was a linear fluorescence at the DE junction. However, at places where amyloid was deposited, a reticular fine network of positive fluorescence was seen in the dermal papillae (Fig 7A,*B*). Fragments or reticular network of elastic fibers, identified by blue autofluorescence, were frequently reduced or absent in the dermal papilla.

A blocking test and the other specimens using FITC-labeled antiserum to human IgG, IgM, C1q, and C3 were all negative.

DISCUSSION

Basal lamina-anchoring fibril complexes were frequently found between and/or within the amyloid islands in the dermal papillae. The basal laminae of blood vessels or nerves do not

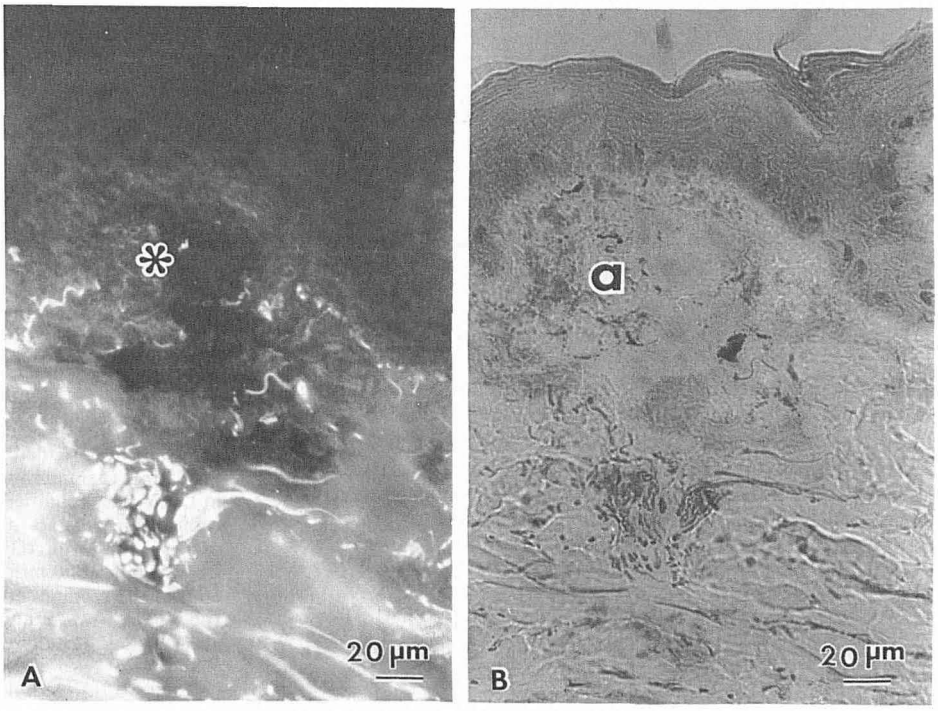

FIG 7. *A*, Indirect immunofluorescence staining of the macular amyloidosis using BP-antibody shows the specific fluorescence in the dermal papillae in a reticular pattern (*asterisk*). *B*, Exactly the same specimen shown in Fig 7*A* was stained with crystal violet. Amyloid (*a*) is seen in the area where the reticular pattern of immunofluorescence was observed.

have anchoring fibrils [9,10]; therefore the fragments must have originated from the basal lamina of the DE junction. The presence of a basal lamina component in this complex was also confirmed by immunohistochemical studies using bullous pemphigoid antibody. Histochemical studies revealed that PAS reaction, presumably staining this complex, was linearly positive in dermal papillae, whereas amyloid deposits were weakly stained as globular masses within the papillae. Widened or entangled basal lamina, as was shown by electron microscopy in this study, seemed to contribute to the linear positivity in the PAS reaction.

The basal lamina-anchoring fibril complexes within the amyloid deposits were considered to be isolated from the DE junction and situated within the dermis because of the characteristic morphology and the localization. In the normal skin, anchoring fibrils were seen in the dermis below the continuous basal lamina [10], whereas anchoring fibrils revealed in this study were between and/or within the amyloid deposits situated in the dermal papillae. The anchoring fibrils were encircled completely by the basal lamina-like substance (bull's-eye structure). They were also surrounded by the arch of homogenous substance. This morphologic variety was not recorded in the normal skin [10]. In addition, the basal lamina at the DE junction frequently showed the features of a newly formed basal lamina—a thin basal lamina without any appreciable anchoring fibrils [10].

The multiplication or fragmentation of basal lamina has been demonstrated in various dermatoses which cause degenerative changes in DE junctions, e.g., systemic and discoid lupus erythematosus [11], lichen planus, dystrophic epidermolysis bullosa, repeated ultraviolet-A irradiation [12], psoralen + ultraviolet-A therapy [13], etc. Therefore, "dropping off" of degenerated epidermal cells through the DE junction might stimulate the repair mechanism and induce multiplication of basal laminae and anchoring fibrils. Another mechanism might be that amyloid mass itself in the subepidermal location stimulated proliferation of basal laminae and anchoring fibrils.

Hyperpigmentation of the amyloid lesions such as "rippling pigmentation" of macular amyloidosis may be explained by this "dropping off" phenomenon or apoptosis of either melanosome-containing degenerated keratinocytes or melanocytes themselves.

Amyloid deposits accompanying epithelial neoplasms, such as in Bowen's disease, basal cell epithelioma, seborrheic keratosis, etc., also could be explained by the same mechanism.

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